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SE Baer
Virginia Institute of Marine Science

TL Connelly

RE Sipler
Virginia Institute of Marine Science

PL Yager

DA Bronk
Virginia Institute of Marine Science

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Key Points:
- Nitrification dominates ammonium sinks during winter and spring
- Ammonium uptake, but not nitrification, increases with warming
- Bacterial production has multiple temperature optima

Correspondence to:
S. E. Baer,
sbaer@bigelow.org

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Effect of temperature on rates of ammonium uptake and nitrification in the western coastal Arctic during winter, spring, and summer

Steven E. Baer[1,2], Tara L. Connelly[3], Rachel E. Sipler[1], Patricia L. Yager[4], and Deborah A. Bronk[1]

[1]Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, Virginia, USA, [2]Now at Bigelow Laboratory for Ocean Sciences, East Boothbay, Maine, USA, [3]Marine Sciences Institute, University of Texas at Austin, Port Aransas, Texas, USA, [4]Department of Marine Sciences, University of Georgia, Athens, Georgia, USA

Abstract Biogeochemical rate processes in the Arctic are not currently well constrained, and there is very limited information on how rates may change as the region warms. Here we present data on the sensitivity of ammonium (NH_4^+) uptake and nitrification rates to short-term warming. Samples were collected from the Chukchi Sea off the coast of Barrow, Alaska, during winter, spring, and summer and incubated for 24 h in the dark with additions of $^{15}NH_4^+$ at −1.5, 6, 13, and 20°C. Rates of NH_4^+ uptake and nitrification were measured in conjunction with bacterial production. In all seasons, NH_4^+ uptake rates were highest at temperatures similar to current summertime conditions but dropped off with increased warming, indicative of psychrophilic (i.e., cold-loving) microbial communities. In contrast, nitrification rates were less sensitive to temperature and were higher in winter and spring compared to summer. These findings suggest that as the Arctic coastal ecosystem continues to warm, NH_4^+ assimilation may become increasingly important, relative to nitrification, although the magnitude of NH_4^+ assimilation would be still be lower than nitrification.

1. Introduction

The Chukchi Sea receives significant nutrient inputs from the North Pacific Ocean [*Codispoti et al*., 2005]. Yet the coastal shelves of this region have high rates of denitrification [*Devol et al*., 1997] and likely exhibit overall nitrogen (N) limitation of phytoplankton growth [*Codispoti et al*., 2009]. In the very shallow, nearshore area, there is a lack of established baseline data for N uptake rate measurements, especially during the dark and frigid winter months [*Codispoti et al*., 2005]. There is an urgent need to understand N uptake and nitrification in the Arctic, as the region is warming faster than almost anywhere else on the planet [e.g., *Serreze and Francis*, 2006]. This warming has already caused reductions in sea ice extent and volume [*Stroeve et al*., 2007], freshening of the surface ocean [*Yamamoto-Kawai et al*., 2009], and numerous other impacts on the overall ecology of the system [*Grebmeier*, 2011; *Wassmann et al*., 2011]. The nutrient regime of the Arctic is also changing, with decreasing Pacific inputs [*Codispoti et al*., 2009] and increasing terrestrial runoff [*Peterson et al*., 2002]. Uptake and regeneration of ammonium (NH_4^+) play a key role in the structure and productivity of ecosystems. When N is limiting, microbial community structure and function depend in part on whether NH_4^+ is assimilated directly into biomass or instead converted to nitrate (NO_3^-) via nitrification. It is not clear how these relative rates will change as the climate warms, especially at high latitudes.

Although published reports of pelagic nitrification in the Arctic are limited [*Deal et al*., 2011], there are numerous reports of nitrifying organisms in Arctic waters [*Alonso-Sáez et al*., 2012; *Bano and Hollibaugh*, 2000; *Galand et al*., 2009; *Kalanetra et al*., 2009]. The one study that has reported a seasonal comparison of nitrification measurements in coastal Arctic waters found much higher rates during winter than summer and attributed this to the chemoautotrophic potential of the prokaryotic community during the cold and dark conditions under sea ice [*Christman et al*., 2011]. In the euphotic zone, light is thought to inhibit nitrification [*Ward et al*., 1984], but recent evidence suggests this to be highly equivocal [*Yool et al*., 2007]. Even if nitrification is inhibited by light, it may depend on which wavelength [*Guerrero and Jones*, 1996] or whether the dominant taxa are archaeal or bacterial nitrifiers [*Merbt et al*., 2012]. *Tremblay et al*. [2008] hypothesized that winter nitrification, rather than physical processes, could be the cause of consistently high NO_3^- concentrations in the Arctic Ocean during winter and spring preceding the spring bloom period.

Table 1. Chemical and Biological Parameters for Samples Taken From the Coastal Arctic in Winter, Spring, and Summer[a]

Date	Station	Sample Depth (m)	Ambient Water Temperature (°C)	NH_4^+ (μmol N L^{-1})	NO_2^- (μmol N L^{-1})	NO_3^- (μmol N L^{-1})	DOC (μmol C L^{-1})	Chl *a* (ug L^{-1})	Bacterial Abundance (10^8 cells L^{-1})
					2011				
30 Jan	WIN1	2	−1.9	3.07	BDL	7.66	74.0	0.03	4.6 ± 0.2
26 Apr	SPR1	6.5	−1.6	0.81	BDL	8.41	67.9	0.11	2.6 ± 0.1
28 Apr	SPR2	6.5	−1.6	0.51	BDL	8.57	67.6	0.10	2.1 ± 0.2
30 Apr	SPR3	4	−1.8	1.25	BDL	11.4	67.2	0.06	3.9 ± 0.5
17 Aug	SUM1	4	+4.7	0.59	BDL	0.32	93.8	0.37	18 ± 0.5
18 Aug	SUM2	2	+4.7	0.47	BDL	0.33	93.7	0.62	15 ± 0.3
					2012				
16 Jan	WIN2	2	−1.8	0.60	BDL	11.7	82.3	0.03	2.5 ± 0.1
19 Jan	WIN3	1	−1.8	0.96	0.05	9.86	85.7	0.01	2.9 ± 0.5

[a]Ambient water temperature and nutrient and plankton concentrations at the time of water collection for each experiment and season. BDL is for below detection limit (0.03 μmol N L^{-1}). DOC, dissolved organic C; Chl *a*, chlorophyll *a*.

As the Arctic continues to warm and the ice-free season expands, our understanding of how the rates of NH_4^+ uptake and nitrification may change is not well constrained, especially during seasons other than summer. It is generally expected that rate processes respond positively to temperature increases, although a recent meta-analysis indicated that bacterial activity is not more sensitive to temperature increases in the polar regions as compared to the temperate zone; rather, the increased dissolved organic matter (DOM) supply in the Arctic can explain most of the large increase in rates reported at low temperatures [*Kirchman et al.*, 2009]. Synergy between temperature sensitivity and substrate concentration in Arctic marine systems has been reported for organic N compounds, such that some microorganisms have greater substrate demands at lower temperatures [*Wiebe et al.*, 1992; *Yager and Deming*, 1999]. There is limited information on the impact of low temperatures and for inorganic N substrates on primary and bacterial production. In a culture study of psychrophiles, there is evidence of NO_3^-, but not NH_4^+, uptake being hampered at low temperatures [*Reay et al.*, 1999]. Antarctic sea ice algae had maximum uptake rates of inorganic N between 0.5 and 3.0°C, below which both NO_3^- and NH_4^+ uptake decreased by at least half [*Priscu et al.*, 1989].

In this study, we tested the sensitivity of NH_4^+ uptake and nitrification rates to warming in nearshore Chukchi Sea waters by incubating winter, spring, and summer seawater samples under a range of temperatures. This study provides a current baseline for these processes along with insight into how they may change under the specter of future warming.

2. Materials and Methods

Sampling and analytical methods were part of a larger study investigating overall N uptake and regeneration in the coastal Chukchi Sea near Barrow, Alaska. Briefly, coastal seawater was sampled during January, April, and August 2011, and again during January 2012. Experiments were performed multiple times during each trip, with the exception of the first winter (Table 1). All of the sampling was done at approximately the same location (71°21′N, 156°41′W) with a bottom depth of 17 m, except for the very last sampling event (WIN3), when dangerous ice conditions forced us to move approximately 1 km northeast to a shallower site (71°21′N, 156°34′W; 6 m maximum depth).

Winter and spring samples were collected by traveling to the outer edge of the fast ice by snow machine and then drilling through the ice to sample the seawater below (Table 1). Summer samples were collected from a small boat. A low-pressure electric bilge pump (Johnson Pump) was used to gently draw water through acid-washed and seawater-seasoned 1.25 inch ID Tygon® tubing (Saint Gobain Performance Plastics) into 500 mL acid-washed PETG bottles and then inoculated with 0.2 μmol N L^{-1} additions of ^{15}N-labeled ammonium chloride (98.85% ^{15}N NH_4Cl; Cambridge Isotope Laboratories) and 170 μmol C L^{-1} ^{13}C-labeled sodium bicarbonate (99% ^{13}C $NaHCO_3^-$; Cambridge Isotope Laboratories). An additional set of incubations was performed with additions of 0.2 μmol N L^{-1} of ^{15}N-labeled sodium nitrite (98% ^{15}N $NaNO_2$; Cambridge Isotope Laboratories). The $^{15}NH_4^+$ incubations averaged 17.8 ± 7.7 atom percent enrichment over all seasons, while nitrite (NO_2^-) incubations were essentially 100% (mean of 93.0 ± 8.0) enrichment due to the general lack of measurable NO_2^- concentrations in the water column (Table 1).

After inoculation, bottles were insulated and transported back to the laboratory; temperature changes during transport were limited to less than 0.3°C during all seasons. Upon return to the lab, duplicate bottles were placed in dark water baths for 24 h at −1.5, 6, 13, and 20°C; digital thermometer probes were used to continuously monitor incubation temperatures. The in situ mean water temperature in summer was +4.7°C so the lowest temperature incubation (i.e., −1.5°C) actually represents cooling of the sample (Table 1). In situ water temperature for both winter and spring was approximately −1.8°C. At the end of the incubation, samples were filtered through Whatman GF/F (0.7 μm nominal pore size) filters, which retained 34, 74, and 61% of the bacterial cells during winter, spring, and summer, respectively [*Baer*, 2013]. The filters were placed in cryovials and the filtrate into polypropylene tubes and frozen until analysis.

Bacterial production was measured using the leucine incorporation method [*Ducklow*, 2000; *Kirchman*, 2001; *Smith and Azam*, 1992] and was measured on the initial sample and following each treatment by incubating triplicate 1.5 mL aliquots with tritiated leucine (^{3}H-leu; specific activity of 144 Ci mmol^{-1}) at a final concentration of 25 nM for 4 h in the dark. Incubations were terminated by adding 0.1 mL of 100% trichloroacetic acid (TCA) to each sample tube. Samples were centrifuged, and protein was extracted by rinsing the samples again with 1 mL of ice-cold TCA and then by rinsing with 1 mL of 80% ethyl alcohol, with centrifugation between each rinse. After placement in a fume hood overnight to dry, 1 mL of UltimaGold™ scintillation cocktail (PerkinElmer) was added to each tube, and the radioactivity was measured on a liquid scintillation counter. Killed controls were performed by adding the ^{3}H-leu after killing the cells with the addition of TCA. For two of the experiments (WIN1 and SPR1), we use the bacterial production results from a parallel set of incubations performed on the same water but without ^{15}N tracer added. In August 2011, no radioisotope was available, but bacterial production rates were measured at the same site during August 2010 and are used here for comparison to the seasonal production rates.

Bacterial abundance was measured in triplicate from 3.6 mL samples that were fixed with 400 μL of formaldehyde and refrigerated at 6°C for 15 min to allow complete fixation. They were subsequently frozen at −80°C until analysis. After staining with SYBR Green (Invitrogen) and the addition of reference beads (Spherotech, Fluorescent Yellow Particles, 1.7–2.2 μm), samples were run in duplicate on a FACScalibur flow cytometer (Becton Dickinson) and analyzed using FlowJo software (Treestar Inc.).

Concentration of NH_4^+ was measured in triplicate using the phenol-hypochlorite method [*Koroleff*, 1983]. Concentrations of NO_3^- and NO_2^- were measured in duplicate using a Lachat QuikChem 8500 autoanalyzer [*Parsons et al*., 1984]. All ^{15}N and ^{13}C uptake samples were run on a Europa GEO 20/20 mass spectrometer with an automated nitrogen carbon analysis autosampler. We used the calculations of *Dugdale and Goering* [1967] and *Hama et al*. [1983] for N and carbon (C) uptake rates, respectively. Solid phase extraction [*Brzezinski*, 1987; *Dudek et al*., 1986] was used to isolate the NH_4^+ pool so that the final NH_4^+ atom percent (at. %) could be determined and used to correct for isotope dilution in calculations [*Glibert et al*., 1982]. The denitrifier method [*Sigman et al*., 2001] was used to determine the at. % of the NO_3^- pool. Rates of nitrification were calculated by tracing the labeled N from NH_4^+ into the NO_2^- and NO_3^- pools (NO_x) collectively.

$$\text{nitrification} = \frac{\text{at. \% NO}_x}{\text{at. \% NH}_4^+ \times \text{time}} \times [\text{NO}_x] \quad (1)$$

The at. % of each substrate was corrected to at. % normal by subtraction of 0.3667. Time in this equation is the total time of the incubation, and the NO_x concentration is at the end of the incubation.

To assess the impact of temperature on the rates, Q_{10} values, which estimates how much a biological rate increases with a 10°C rise in temperature, were calculated for each positive change in rate at each temperature difference, by the following equation:

$$Q_{10} = \left(\frac{\text{rate}_2}{\text{rate}_1}\right)^{\frac{10}{T_2 - T_1}} \quad (2)$$

[*Segal*, 1975] where the rate is the measured rate at a specific temperature, T is temperature of the incubation, and the subscripts refer to two distinct temperature incubations. Data were analyzed using two-way analysis of variance and Tukey's Honestly Significant Difference method. Differences were considered significant at a p value < 0.05, while correlation coefficients (R values) ≥ 0.4 were considered significant.

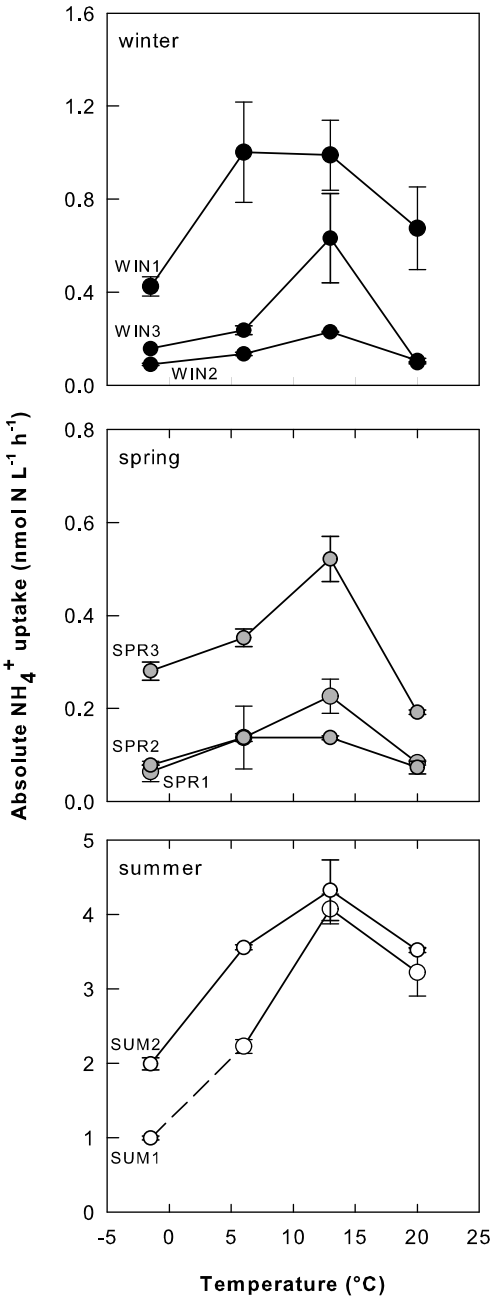

Figure 1. Ammonium uptake rates in the coastal Arctic during winter, spring, and summer. The station IDs for each experiment, as defined in the text and Table 1, are given next to each plot. Error bars are standard deviation. Note different *y* axis scales. The SUM1 −1.5°C treatment was not corrected for isotope dilution, as indicated by the dashed line.

3. Results

3.1. Ambient Conditions

Water temperature during winter and spring was consistently −1.8°C, and in summer, it was 4.7°C. Nutrient concentrations, however, were highly variable from season to season and year to year. During January 2011, for example, the ambient NH_4^+ concentration was 3.1–5.1 times higher than during January 2012 (Table 1). The seasonal minimum in both NH_4^+ and NO_3^- concentrations occurred during summer, and there was a much smaller range in NH_4^+ concentrations than NO_3^- concentrations. The NO_3^- concentrations during summer were 0.4 μmol N L^{-1}, which is < 5% of concentrations measured during winter and spring (Table 1). NO_2^- was only detectable (limit = 0.03 μmol N L^{-1}) during one winter station (WIN3), and even then it was only 0.05 μmol N L^{-1}. Bacterial abundance during winter and spring had a twofold range (2.1–4.6 × 10^8 cells L^{-1}; Table 1) and were 3–8 times higher (15–18 × 10^8 cells L^{-1}) in summer.

3.2. Uptake of Nitrogen and Carbon

During January 2011 (WIN1), NH_4^+ uptake rates peaked at 6°C and plateaued to a statistically equal value at 13°C, with a slight decrease at 20°C (Figure 1). Calculations of Q_{10} reflect a strong sensitivity to temperature from −1.5 to 6°C (Table 2). The January 2012 experiments (WIN2 and WIN3) both had maxima at 13°C. When the ambient NH_4^+ concentration was 3 times higher (i.e., WIN1), the uptake rate at in situ temperature (−1.5°C) was more than double that of 2012 (WIN3) for all temperatures except 13°C (Figure 1). During spring, one station (SPR3) had a strikingly similar pattern of NH_4^+ uptake to winter 2012 (WIN2 and WIN3) but at slightly reduced rates (Figure 1). Uptake rates at SPR1 and SPR2 peaked at statistically equal rates ($p < 0.05$) in the 6 and 13°C incubations.

During summer, uptake rates were significantly higher, but the effect of warming was similar (Figure 1). The peak NH_4^+ uptake rate was measured at 13°C and was significantly different from −1.5°C ($p < 0.001$). The Q_{10} values were highest for the −1.5 to 6°C change during summer and lower for the 6 to 13 and −1 to 13°C temperature increases. Although the initial rate (i.e., at ambient temperature) was lower during SUM1, there was a greater warming response reflected in the higher Q_{10} values.

The different temperature treatments and N additions did not cause a relative increase in bacterial abundance or particulate N (data not shown). Additionally, cell-specific uptake rates of NH_4^+ (data not shown) had a similar relative pattern to the whole community rates. These factors indicate that the change in rates is likely a physiological response, and not due to any short-term increases in biomass.

Table 2. Q_{10} Values From the Coastal Arctic in Winter, Spring, and Summer[a]

	2011						2012	
	Spring			Summer		Winter	Winter	
	SPR1	SPR2	SPR3	SUM1	SUM2	WIN1	WIN2	WIN3
				NH_4^+ Uptake				
−1.5 to 6°C	3.0 ± 0.6	2.2 ± 0.1	1.4 ± 0.1	3.2 ± 0.1	2.3 ± 0.0	2.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
6 to 13	2.1 ± 0.5	1.0 ± 0.1	1.8 ± 0.1	2.4 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	2.2 ± 0.1	5.3 ± 0.1
−1.5 to 13	2.5 ± 0.4	1.5 ± 0.0	1.6 ± 0.1	2.7 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	3.1 ± 0.0
				Nitrification				
−1.5 to 6°C	0.9 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	ND	1.2 ± 0.1	1.2 ± 0.0	1.0 ± 0.0	1.1 ± 0.0
6 to 13	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.0 ± 0.0	0.8 ± 0.0	1.0 ± 0.0	1.1 ± 0.0
−1.5 to 13	1.0 ± 0.1	1.1 ± 0.0	1.0 ± 0.0	ND	1.1 ± 0.1	1.0 ± 0.0	1.0 ± 0.1	1.1 ± 0.0
				Bacterial Production				
−1.5 to 6°C	*2.2 ± 0.1*	ND	*3.1 ± 0.3*	*1.6* ± 0.1	ND	2.3 ± 0.1	2.9 ± 0.1	2.3 ± 0.0
6 to 13	*2.3 ± 0.0*	ND	*1.3 ± 0.3*	*3.4* ± 0.1	ND	1.7 ± 0.1	4.6 ± 0.2	1.9 ± 0.1
−1.5 to 13	*2.2 ± 0.1*	ND	*2.0 ± 0.3*	*2.6* ± 0.1	ND	2.0 ± 0.1	3.6 ± 0.1	2.1 ± 0.1

[a] Q_{10} and standard deviation for NH_4^+ uptake, nitrification, and bacterial production rates for each experiment. Values do not include the 20°C treatment, as all experiments had equal or decreased rates at that temperature. The stations are as described in the text. Bacterial production Q_{10} data in italics for SUM1, SPR1 are from separate experiments performed during the same season, as explained in section 2. ND is for no data.

Consistent with the low ambient concentrations of NO_2^-, NO_2^- uptake rates were extremely low. The mean uptake rates at ambient temperature were 0.004 ± 0.002, 0.003 ± 0.000, and 0.043 ± 0.032 nmol L^{-1} h^{-1} for winter, spring, and summer, respectively. There was no discernible pattern to NO_2^- uptake with changes in temperature, although the mean values for winter stations seemed to peak at 13°C and summer stations peaked at either 13 or 20°C but were not significantly different from the other temperatures.

Similarly, HCO_3^- uptake was not measurable during winter. Since incubations were performed in the dark, HCO_3^- uptake rates that we did find are likely due to either a delay in the shutdown of the C fixation process or chemoautotrophic processes (discussed below). Uptake of HCO_3^- during summer displayed two maxima at the lowest and highest temperatures, with a minimum at the intermediate temperatures near in situ (+4.7°C). Mean rates for the summer were 1.57 ± 0.46, 0.82 ± 0.18, 0.89 ± 0.07, and 1.58 ± 0.87 nmol C L^{-1} h^{-1} for the −1.5, 6, 13, and 20°C treatments, respectively.

3.3. Nitrification

During the ice-covered seasons of winter and spring, nitrification rates had no statistically significant temperature maxima for any season (Figure 2) but were much higher than NH_4^+ uptake rates. Overall, nitrification rates were 2 orders of magnitude higher during winter and spring than summer and appear to be sensitive to high concentrations of NH_4^+, as WIN1 and SPR3 both had much higher relative rates that correlate to higher ambient NH_4^+ concentrations (Table 1). Q_{10} values for nitrification emphasize the lack of a temperature effect, being approximately 1 for all temperature differences during winter and spring (range = 0.8–1.2; Table 2). Although one summer station (SUM1) had a slight peak at 13°C and slightly higher Q_{10} values for the lower two temperature ranges, the actual rate of nitrification during the summer was so small that any increase would likely have little impact on ecological processes. When we calculate the assimilation and nitrification rates over the length of the incubation, there would still be enough NH_4^+ in the bottles to measure increased nitrification rates at all temperatures, and we conclude that NH_4^+ supplies did not limit nitrification rates.

3.4. Bacterial Production

Leucine incorporation rates followed a similar pattern to NH_4^+ uptake rates. During winter and spring, there was a peak at 13°C after which the rate declined (Figure 3). The mean Q_{10} for the −1.5 to 6°C and the 6 to 13°C range were not significantly different from each other during either winter or spring (Table 2). Bacterial production at station WIN3 was only slightly less than the spring mean. This is an interesting contrast to the results for the NH_4^+ uptake and nitrification and may be due to sampling at a shallower site closer to the

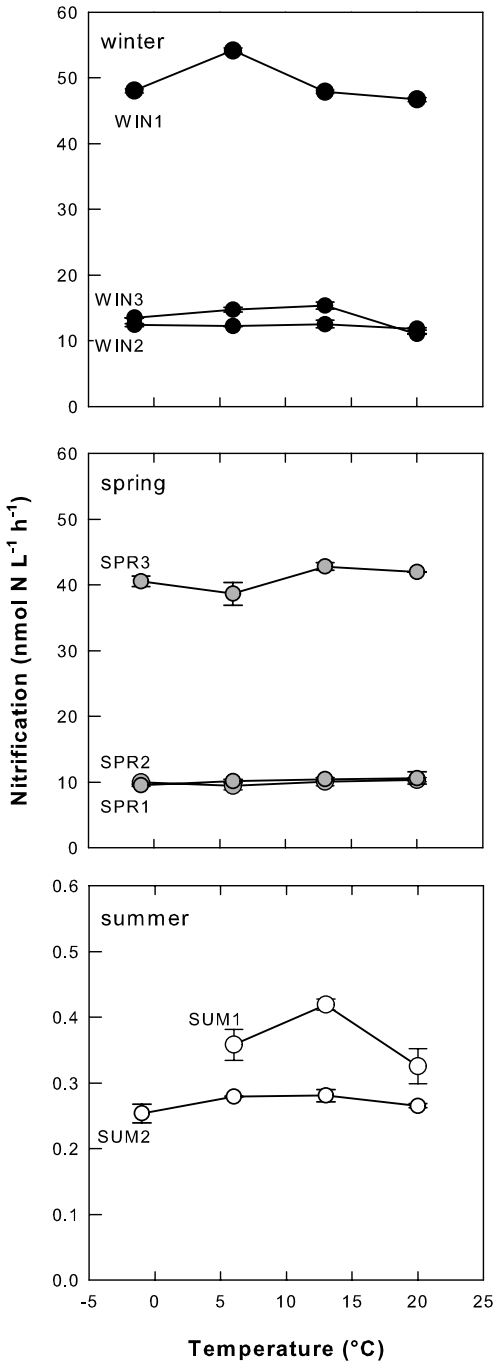

Figure 2. Nitrification rates in the coastal Arctic during winter, spring, and summer. The station IDs for each experiment, as defined in the text and Table 1, are given next to each plot. SUM1 has no data for the −1.5°C treatment, as explained in section 3. Note that the *y* axis scale for the summer plot is 2 orders of magnitude lower than the winter and spring. Error bars are standard deviation.

Beaufort Sea, which had higher dissolved organic C (DOC) concentrations. We found that no stimulation of bacterial production due to the tracer additions compared to our controls that had no ^{15}N substrates added (data not shown).

4. Discussion

Microbes have adapted to many extreme environments that seem to test the limits of survival [*D'Amico et al.*, 2006]. In extremely cold domains, the uptake of substrates at low temperatures (−1.5 to 4°C) can be significantly reduced due to decreased cell membrane fluidity [*Nedwell*, 1999]. To counteract this limitation, an increase in substrate concentrations can overcome the negative effect of low temperature on both mesophilic and psychrotolerant bacteria [*Wiebe et al.*, 1992], although this effect is not always observed [*Kirchman et al.*, 2005, 2009; *Vaquer-Sunyer et al.*, 2010; *Yager and Deming*, 1999]. In this study, we used short-term warming experiments to quantify the extent to which Arctic microplankton in their natural setting can utilize NH_4^+ over a seasonal cycle and how they may respond to warming.

4.1. Ammonium Uptake

Laboratory studies of pure cultures report microbes with low temperature optima, but found that NH_4^+ uptake was not temperature sensitive [*Reay et al.*, 1999]. However, using a meta-analysis of environmental samples of dark NH_4^+ uptake, *Smith and Harrison* [1991] found Q_{10} values greater than 2 for polar regions. Additionally, Antarctic sea ice algae were found to have low-temperature maxima for N uptake and an extraordinarily high Q_{10} of 15.7 for NH_4^+ uptake between 2.0 and 3.0°C [*Priscu et al.*, 1989]. Our results generally show the greatest increase for the −1.5 to 6°C temperature change, with a Q_{10} range of 1.4–3.2 (Table 2) and highest uptake rate at 13°C (Figure 1). The only exception was the winter 2012 season when the maximum sensitivity was found between the 6 and 13°C incubations.

While the community as a whole responded to increases in temperature, there was also an underlying signal of an enhanced response to increased ambient NH_4^+ concentrations, with a potential for synergistic responses if both factors change in the future. Both the NH_4^+ absolute uptake rates and the rate of increase with temperature were positively correlated with higher NH_4^+ concentrations at the beginning of the incubations. Reductions in ice extent and volume could lead to earlier increases in nutrient supply from the receding ice edge, changing the timing of phytoplankton blooms, and increasing and extending the time of dependence on regenerated production [*Wassmann and Reigstad*, 2011]. In nearshore environments, these impacts have the potential to be exacerbated due to changes in the

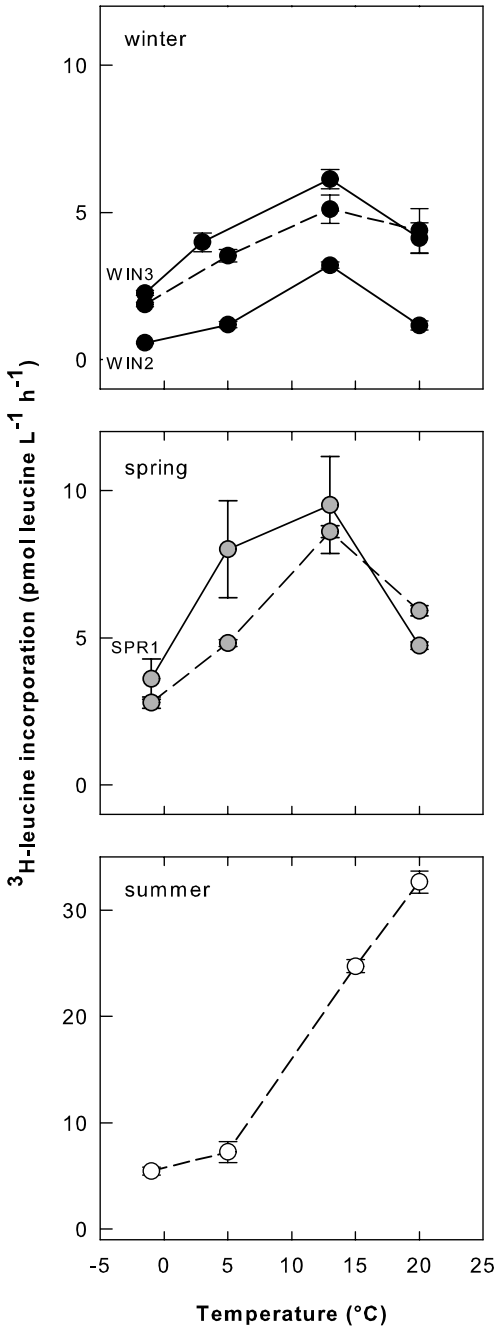

Figure 3. Bacterial production rates in the nitrogen uptake incubation experiments from coastal Arctic waters. The station IDs for each experiment, as defined in the text and Table 1, are given next to each plot. Error bars are standard deviation. Plots with dashed lines are for bacterial production experiments performed in separate incubations (see section 2) and are not labeled with a station name. Note the different *y* axis scale for summer. Error bars are standard deviation.

timing and biological availability of terrestrial runoff resulting from earlier melt [*Peterson et al*., 2002; *Tank et al*., 2012]. This is especially true in the Arctic, where the continental shelves dominate the basin.

4.2. Nitrification

Our rates were determined from additions of NH_4^+ tracked into isotopically labeled NO_3^- at the end of the incubations. We attempted to also quantify the first step in nitrification (NH_4^+ to NO_2^-), but NO_2^- concentrations were below detection ($<0.03\ \mu mol\ N\ L^{-1}$) in most samples. As an intermediary in the nitrification process, the presence of NO_2^- can indicate net NH_4^+ oxidation. Its absence, however, can indicate tightly coupled NH_4^+ and NO_2^- oxidation or an absence of nitrification all together. We hypothesize that the low or undetectable NO_2^- concentrations in this study indicate the former. The first step in the nitrification process will be an avenue for further research. This does not invalidate our results, however, as we were still able to measure the overall nitrification process.

Consistent with other seasonally and perennially cold oceanic regions, nitrification rates were much higher than NH_4^+ uptake rates in winter and spring. During those ice-covered seasons, nitrification dominated NH_4^+ sinks (defined as nitrification and NH_4^+ assimilation), accounting for 99% of NH_4^+ loss. This relationship holds even though there was a large difference in the year-to-year NH_4^+ concentrations and uptake rates during winter. For example, our highest winter nitrification rates ($0.76\ nmol\ N\ L^{-1}\ h^{-1}$) were lower than other cold water sites where dark incubations were performed. A North Sea winter experiment (range = 41–221 $nmol\ N\ L^{-1}\ h^{-1}$), where the conditions included higher temperatures and NH_4^+ concentrations than our study [*Veuger et al*., 2013], still found the predominant sink for NH_4^+ to be nitrification. Similarly, our summer nitrification rate measurements (mean of $2.9 \pm 0.9\ nmol\ N\ L^{-1}\ h^{-1}$ at 5°C) were lower than nitrification rates measured in the perennially cold waters of the Southern Ocean, but where NH_4^+ concentrations were higher (4–10 μM) than our study [*Bianchi et al*., 1997]. During summer, the situation reverses, with NH_4^+ assimilation representing 91% of consumption processes in our study, which aligns with the general trend from temperate areas where nitrification and assimilation have been measured simultaneously [*Lipschultz et al*., 1986; *Ward*, 2005]. Since our results were generated from dark incubations, even these low rates of nitrification are possibly inflated. The interactive effects of light and temperature remain an opportunity for future research. While we found nitrification rates that were exceedingly low and unchanging with temperature, NH_4^+ uptake was significantly higher during the summer compared to the other seasons.

Psychrophiles are known to nitrify at rates comparable to temperate mesophiles, but strong relationships between nitrification and environmental or ecological factors in the water column have yet to be established [*Ward*, 2008]. Phytoplankton can outcompete nitrifiers for NH_4^+ in well-lit ocean layers [*Martens-Habbena et al*., 2009; *Ward*, 2005] and that is likely the case during the polar summer. During the winter, when primary production is light limited, there is reduced competition from bacterial and archaeal N demand. In addition to light-driven competition limits, temperature is often suggested to be a controlling environmental factor, at least for organic N [*Pomeroy et al*., 1990, 1991; *Wiebe et al*., 1992]. Conversely, polar microorganisms are generally cold adapted, and polar bacteria only sometimes exhibit sensitivity to organic substrate availability as temperature decreases [*Yager and Deming*, 1999]. Uptake of inorganic N may exhibit a similar sensitivity. Our results confirm that N uptake at low substrate concentrations is less sensitive to warming than when substrate concentrations are higher. There is potentially a varied biological response to the environmental conditions. More cold-tolerant members of the polar microbial communities may require higher substrate concentrations [e.g., *Wiebe et al*., 1992], whereas the true psychrophiles are less sensitive to substrate concentrations [*Yager and Deming*, 1999].

While there are no other studies that we know of testing the sensitivity of nitrification to temperature in pelagic polar systems, one ammonia oxidizer capable of growth down to −5°C has been isolated from southern Alaskan waters (sub-Arctic Pacific). Even when acclimated to low temperatures, this organism had an optimum temperature for nitrification of 22°C [*Jones et al*., 1988] and therefore is probably not representative of the community present at our Chukchi Sea site. A recent set of experiments in Puget Sound found no change in nitrification rates in incubations ranging from 8 to 20°C [*Horak et al*., 2013]. In the Southern Ocean, *Bianchi et al*. [1997] also performed dark incubations and found no correlation between nitrification rates and temperature nor ambient NH_4^+ concentrations. Nitrification rates have been subjected to warming experiments in Arctic marine sediments and terrestrial soils, the latter of which are warming at an alarming rate. In Arctic marine sediments, optimum temperatures for nitrification are very low, and rates decrease markedly when subjected to experimental warming [*Thamdrup and Fleischer*, 1998]. In Arctic soils, nitrifiers only responded to a temperature change above 10°C, at which point there was a twofold increase in N mineralization [*Nadelhoffer et al*., 1991]. Additionally, warming of Arctic soils has been shown to cause changes in nitrifier community structure [*Avrahami and Conrad*, 2003]. Much like our study, the temperature manipulations in those experiments are above the current normal range but could portend a future in which nitrifier community shifts trigger rapid changes in NH_4^+ assimilation rates.

Over the seasonal cycle, the microbial community itself is already known to change. Bacterial nitrifiers have been found during summer in Arctic surface waters [*Hollibaugh et al*., 2002]. During the Arctic winter, there is high crenarchaeal abundance [*Alonso-Sáez et al*., 2008], and these organisms are known nitrifiers both in the Arctic Ocean [*Christman et al*., 2011] and in other oceanic realms [*Grzymski et al*., 2012; *Wuchter et al*., 2006]. Crenarchaeota have been found with high affinities for NH_4^+ at both low [*Martens-Habbena et al*., 2009] and high [*Morris et al*., 2010] substrate concentrations and have even recently been shown to utilize organic N compounds during the Arctic winter [*Alonso-Sáez et al*., 2012]. It has been proposed that Crenarchaea are responsible for high winter rates seen in this region, with ammonium monooxygenase gene (*amoA*, which encodes the enzyme responsible for the first step in the nitrification process) copy numbers to be positively correlated with both the winter season and increasing NH_4^+ concentration [*Christman et al*., 2011].

The aforementioned environmental factors (light, competition, substrate, and community composition) all combine to favor the use of NH_4^+ as an energy source (i.e., nitrification) during winter and spring in the Arctic. Short-term warming of the native seasonal communities did not impact rates of nitrification but did increase NH_4^+ uptake rates. Long-term microbial community shifts in response to warming would likely exacerbate the results found here. With warming, marine microbial communities are expected to undergo changes in cell size structure [*Daufresne et al*., 2009; *Morán et al*., 2010], biogeography [*Falkowski and Oliver*, 2007], and food web dynamics [*O'Connor et al*., 2009]. Our sampling conditions necessitated short-term warming incubations. This type of experimental warming may be an underestimation of the community response, as some species may be better at acclimating to changing conditions if given more time, but we were assessing the maximal response to temperature changes. On the other hand, bacteria at high latitudes function well below their optimum temperature [*Pomeroy and Wiebe*, 2001] and will not need any adaptive capacity to adjust to future predicted increases in temperature.

An important caveat to the results presented thus far is the need to perform dark incubations in order to isolate nitrification rates. Although not subjected to warming, a companion study (S. E. Baer et al., unpublished data, 2013) measured NH_4^+ uptake under ambient light conditions. When compared to the seasonal ambient temperature incubations in the dark treatments of this study, winter dark NH_4^+ uptake accounts for 98% of the rate measured in the light. During spring and summer, however, dark NH_4^+ uptake is only 8 and 21% of the overall rate, respectively. So while dark NH_4^+ uptake is dwarfed by nitrification rates during winter and spring, future changes in ocean temperatures could have profound impacts on the Arctic N cycle, especially during the winter season. We acknowledge that this type of extrapolation from one sample site within the Chukchi Sea is tenuous but does provide support for studying NH_4^+ uptake and nitrification on a larger scale throughout the Arctic to gain better resolution and more realistic current and future Arctic N budgets.

4.3. Production

It has been proposed that bacterial production in the polar regions depends more on DOM supply than temperature [*Kirchman et al.*, 2009]. In this experiment, where we artificially warmed the incubations on short time scales, the strongest bacterial response occurred in the first increment above ambient temperature (i.e., −1.5 to 6°C during winter/spring and 6 to 13°C during summer), while the temperature with the highest rate was even higher (13°C for winter and spring). The bacterial community is therefore cold-loving or psychrophilic by traditional definitions [*Morita*, 1966, 1975]. Production increases were more pronounced in the winter of 2011 (WIN1) and the shallow site sampled in winter 2012 (WIN3). DOC was higher during winter than spring, and the highest concentration outside of summer was measured at WIN3 (Table 1), which could provide an explanation for the heightened production during winter. A companion study performed during the summer of the prior year found a consistent rise in bacterial production with temperature, with an optimum ≥20°C (T. L. Connelly, unpublished data, 2014), which is the same or greater relative increase from ambient temperature as the winter and spring incubations in our study (Figure 3).

Summer uptake of HCO_3^- had maxima at both the lower (−1.5°C) and upper (20°C) range of our temperature incubations, even though the ambient water temperature was 4.7°C. It is likely that the overall community contains both psychrophilic and psychrotolerant species, and each of these is responding to the temperature change, just in different ways. Warming would seem to favor the psychrotolerant groups. It is not surprising that we were unable to quantify C fixation during the winter and spring periods, as autotrophic production would ostensibly be very limited in the absence of light. On the other hand, chemoautotrophic organisms are likely present under sea ice [*Christman et al.*, 2011; *Kirchman et al.*, 2007], and one study reports HCO_3^- uptake by heterotrophs during the dark Arctic winter [*Alonso-Sáez et al.*, 2010]. The fact that we did not observe dark C fixation in our study could indicate its absence, could be due to bacterial cells passing through the GF/F filters or that we did not add enough ^{13}C label to discern a signal. Evidence for the latter is found in a companion study [*Connelly et al.*, 2014] where HCO_3^- uptake was only discernable via molecular methods during winter in incubations with greater concentration of labeled HCO_3^- and termination on smaller pore size filters (0.45 μm). Using a 35:1 molar conversion of NH_4^+ oxidation to carbon fixation [*Ward*, 2008], we calculate an expected mean HCO_3^- uptake rate of 0.99 nmol C L^{-1} h^{-1} which is within the range of summer rates. Clearly, more work is needed to understand winter C dynamics.

5. Conclusions

The future of the Arctic marine ecosystem is unknown, but the region is certainly changing [*Arctic Climate Impact Assessment (ACIA)*, 2005]. Rising air and water temperatures have already been recorded, along with subsequent losses in sea ice volume [e.g., *Stroeve et al.*, 2007] and its attendant changes in the biogeochemistry and food webs of the Arctic [*Wassmann et al.*, 2011]. While there is some evidence that chlorophyll *a* in the coastal Arctic has been declining during the past century [*Boyce et al.*, 2010], it is expected that more open water conditions will lead to increased annual phytoplankton production [*Arrigo et al.*, 2008] and associated increased importance of DOC and bacterial activity [*Kirchman et al.*, 2009]. In Arctic freshwater systems, higher bacterial and viral activity have already been observed [*Säwström et al.*, 2007], and the marine system could be expected to follow the same trend. Based on the results of our study, we would expect further increases in NH_4^+ uptake during each season in the future, especially if the region warms faster during the summer months than winter and spring [*Wang et al.*, 2012]. As light limitation is lifted earlier in the year [*Maslanik et al.*, 2007],

phenological changes to the phytoplankton community may lead to earlier reduction in bacterial nitrification. While light inhibition of nitrification is equivocal, it is clear that nitrification rates plummet during the Arctic summer months [*Christman et al*., 2011] and that this may be a polar coastal phenomenon [*Grzymski et al*., 2012]. Based on our results, warming will have a disproportionately greater effect on assimilation, relative to nitrification. Regardless of the magnitude of the two rates, if more ammonium is going to assimilation than there will be less available to be nitrified to nitrate. This could ultimately drive the system closer to dependence on NH_4^+ supplies, rather than any NO_3^- built up in the system during the ice-covered seasons.

The results of our nitrification warming experiments are in line with other studies done in a wide variety of different oceanic zones, including temperate coastal and open ocean sites. Working in Puget Sound, *Horak et al*. [2013] also found no change in nitrification rates when a natural community was subjected to warming. It is suspected that nitrification is an important factor in both the global N and C cycles [*Yool et al*., 2007]. While this study was limited to a small area of the coastal Arctic, N biogeochemistry of this region can have outsized impacts on global N cycling, including N budgets in the North Atlantic [*Yamamoto-Kawai et al*., 2006]. Current evidence points to large seasonal differences in nitrification rates, along with future increases in the relative importance of NH_4^+ uptake to nitrification. While more research is needed to tease out the interactive effects of light, temperature, and competition for inorganic substrates, current evidence suggests that future increases in temperature could have far-reaching impacts on global biogeochemical cycles.

Using the data from this study, and assuming a 6°C rise in future temperature (as predicted by midcentury [*ACIA*, 2005]), the increase in NH_4^+ uptake over the top 20 m of the Chuckchi Sea (as defined by *Jakobsson* [2002]) will equate to a monthly total of 74, 55, and 1694 mol N for winter, spring, and summer, respectively. These are difficult numbers to put into context, as there is currently very little data on current rates of NH_4^+ uptake [*Lee and Whitledge*, 2005; *Mulholland and Lomas*, 2008] and nitrification [*Christman et al*., 2011] in the western Arctic, especially outside of summer. Additionally, future changes in nutrient inputs from the North Pacific via the Bering Strait and Arctic freshwater sources are not well constrained. It is generally expected, however, that as the ocean warms and freshens, it will become more stratified, therefore lessening nutrient inputs to the Chukchi Sea [*Li et al*., 2009; *Peterson et al*., 2006]. Warming in the Arctic will coincide with other large-scale changes to the ecosystem. Many of these impacts will likely exacerbate the effects of warming on N uptake and nitrification, with the cumulative effects of change to the marine Arctic likely to result in increased demand and competition for NH_4^+. Reductions in seawater pH will lower rates of nitrification [*Beman et al*., 2011], while increases in light (due to reduced ice cover) will enhance primary production [*Arrigo et al*., 2008] and therefore raise overall demand for N, while simultaneously shifting to preference for regenerated N.

The results of the present study are the first in this region to quantify the impacts of warming on bacterial N cycling processes. Our results show that it is imperative to gain a better grasp on nitrification in the Arctic, which is essential to model both current and future N and C cycling in the Arctic, and likely beyond. We have shown a large disparity in the rate of nitrification during the seasons sampled, with a potential dependence on nutrient supply. Even though we tested a wide range of temperatures, no changes were seen in the nitrification rates. Uptake of NH_4^+ on the other hand is highly sensitive to warming and our results suggest that the biogeochemistry and ecology of the western coastal Arctic will be impacted in the coming decades as the region warms.

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